



Studies on ethanol production from water hyacinth—A review

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ABSTRACT

With industrial development growing rapidly, there is a need for environmentally sustainable energy sources. Ethanol from biomass, bioethanol, is an attractive, sustainable energy fuel source for transportation. Based on the premise that fuel bioethanol can contribute to a cleaner environment and with the implementation of environmental protection laws in many countries, demand for this fuel is increasing. Efficient ethanol production is based on optimized processes where utilization of cheap substrates is highly demanding. Utilization of different types of lignocellulosic materials can be considered for production of ethanol. Among various types of lignocellulosic substances water hyacinth (*Eichhornia crassipes*) is a potential resource available in many tropical regions of the world. It is a noxious aquatic weed which grows fast. A considerable amount of research work is in progress for its bioconversion into ethanol using two-sequential steps of hydrolysis and fermentation. This paper reviews the bioconversion of water hyacinth to ethanol.

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1. Introduction

Global depletion of energy supply due to continuing over-utilization is a major problem of the present and future world community. The continuous depletion of fossil fuel reserves and consequent escalation in their price has stimulated interest in development of alternative technologies and substrates to meet the global energy demand.

Global crude oil production is predicted to decline due to shortage of fossil fuels. The emissions of green house gases and air pollution by incomplete combustion of fossil fuels have also resulted in an increased focus on production of biofuel from lignocellulosic biomass. It is estimated that the fossil fuels will be running out by the next few decades [1,2]. Use of renewable energy worldwide and that the developed countries should decrease the net emission of CO₂ [3] by using renewable sources of energy thereby reducing global warming to some extent.

India lacks sufficient domestic energy resources and must import much of its fossil fuels to satisfy growing energy requirements. India is not only experiencing energy shortage but is also increasingly depends on oil imports to meet its demand. In addition to pursuing domestic oil and gas exploration and production projects, India is also stepping up its natural gas imports, particularly through imports of liquefied natural gas. The country's ability to secure a reliable supply of energy sources at affordable prices will be one of the most important factors in shaping its future energy demand.

Coal accounts for more than half (53%) of India's total energy consumption followed by oil, which comprises 31% of the total energy consumption. Natural gas and hydroelectric power accounts for 8% and 6% of consumption, respectively. According to Indian government, 30% of India's total energy needs are met through imports.

An important reason for interest in renewable energy sources is the concern about greenhouse effect. Ethanol has attracted worldwide attention because of its potential use as an alternative automotive fuel. It has immense importance for countries such as India which depends heavily on import of crude oil, spending a huge sum of its annual budget. Ethanol also has value as oxygenate in clean-burning gasoline to reduce vehicle exhaust emissions. Ethanol has a much higher latent heat of vaporization (855 MJ/kg) than petrol (293 MJ/kg). Ethanol has a higher octane number (99) than petrol (80–100) as a result pre ignition does not occur when ethanol is used. Ethanol is burnt more completely so that hydrocarbon emission is drastically lower as compared to petrol. Ethanol is much less likely to catch fire and explode in case of fuel leakage. Ethanol performs well as a fuel in cars either in neat form or in a mixture with gasoline. In addition to ethanol/gasoline blend markets, ethanol has other motor fuel applications including: (1) use as E85, 85% ethanol and 15% gasoline, (2) use as E100, 100% ethanol with or without a fuel additive, and (3) use in oxy-diesel, typically a blend of 80% diesel fuel, 10% ethanol and 10% additives and blending agents. There are numerous advantages of blending ethanol with gasoline resulting in lower quantities of carbon mono-oxide (CO), nitrogen oxides (NO_x), and hydrocarbon after combustion compared to those of gasoline alone because ethanol acts as oxidizing agent [4–6]. Since the combustion of ethyl alcohol

is smokeless and odourless and the net calorific value is 7100 cal/g, it offers a possibility for its use in small stoves in certain situations. It can be used for lighting purpose also. Ethanol with a chemical formula C₂H₅OH is a key component of alcoholic beverages. It is a colourless, transparent, neutral, volatile, flammable, oxygenated liquid hydrocarbon, which has pungent odour and a sharp burning taste. Because of its relatively high affinity for both water and organic compounds, ethanol has found considerable industrial applications. The important physical properties of ethanol are listed in Table 1.

Development of ethanol as a motor fuel can work to fulfill this commitment. Greenhouse gas emission reductions should be estimated on an annual basis. Where the levels from year to year vary significantly, these should be specified on an annual basis. If bioethanol from biomass is used to drive a light-duty vehicle, the net CO₂ emission is less than 7% of that from the same car using reformulated gasoline.

The traditional feed stocks like molasses, sugarcane juice, corn, etc. are used for ethanol production but have social and economical barriers. Apart from these feed stocks, lignocellulosic biomass which comprise mostly of cellulose (20–50%), hemicellulose (20–35%), and polyphenolic lignin (10–35%) [5,6,8], is an alternative feed stock for bioethanol production. The lignocelluloses are by far, the earth's most prevalent renewable organic materials available for microbial or other conversions. This biomass including forest residues such as wood; agricultural residues such as sugarcane bagasse, corn cob, corn stover, wheat and rice straw, has received widespread interest due to their availability, abundance and relatively low cost [9]. However the main crux to the problem lies in the fact that the separation procedure of lignin is very difficult, work is being carried out in this regard and still some more research is needed in this direction.

Water hyacinth, *Eichhornia crassipes* is a monocotyledonous freshwater aquatic plant, belonging to the family Pontederiaceae, related to the lily family Liliaceae and is a native of Brazil and Equador region. It is a well known ornamental plants found in water gardens and aquariums, bears beautiful blue to lilac coloured flowers along with their round to oblong curved leaves and waxy coated petioles. It grows from a few cm to about a meter in height. The stem and leaves contain air filled sacs, which help them to stay afloat in water. In the developing world, it is used in traditional medicine and even used to remove toxic elements from polluted water bodies. They reproduce both asexually through stolons and sexually through seeds, which remain viable for up to 20 years and therefore are difficult to control. Thus, it is also considered as a noxious weed in many parts of the world as it grows very fast and depletes nutrient and oxygen rapidly from water bodies, adversely affecting flora and fauna. Moreover, due to high evapotranspiration it adds to water crisis all over the places where it grows. The possibility of converting water hyacinth to biogas or fuel ethanol is currently established in a number of developing countries, mainly in India. Production of food, fuels and chemicals from materials considered as “waste” constitutes a valuable service in the self-sustaining society we might envision for the future and fermentation is a possible method to achieve this goal.

Lignocellulosic hydrolysates, however, contain substances that inhibit microbial fermentation to desirable products like:

Table 1
Physical properties of ethanol.

Boiling point (°C, at 101 kPa)	Density at 20 °C	Refractive index (n) at 20 °C	Viscosity at 20 °C (ρ)	Specific heat (cal/g °C)	Evaporation heat (cal/g)	Combustion heat (kcal/mol)	Ignition point (°C)
78.4	0.78510	1.3633	0.0122	0.581	204	328	18.3

Adopted from Food & Industrial Microbiology Industrial Production of ethanol in India [7].

- Mono-aromatic inhibitors which include phenolic compounds formed during the pretreatment process by degradation of lignin.
- Phenolic compounds have been reported to be formed in acidic aqueous solutions of carbohydrates which include wood polysaccharide.

The yeast *Saccharomyces cerevisiae* is relatively resistant to inhibitors, but detoxification may still be necessary in order to reach maximum productivity in the fermentation process. Mono-aromatic inhibitors include phenolic compounds formed during the pretreatment process by degradation of lignin, aromatic plant polymers synthesized from phenylpropanoid precursors. Phenolic compounds also occur in wood as extractives. In addition, a variety of phenolic compounds have been reported to form in acidic aqueous solutions of carbohydrates which include wood polysaccharide components such as D-glucose, D-xylose, L-arabinose, D-glucuronic acid, and D-galacturonic acid at elevated temperatures. White-rot fungi are known for their ability to degrade lignins. One of the best-studied white-rot fungi is the basidiomycete *Trametes versicolor*, which secretes enzymes such as the phenol oxidase (laccase) and peroxidase which take part in the transformation of aromatic compounds.

Research work is going on in this aspect of detoxification to obtain maximum productivity and still more research is needed to develop methods which are more economic and less time consuming. The general process description is shown in Fig. 1.

2. Process for conversion of water hyacinth to ethanol

2.1. Chemicals, reagents and microorganisms

Absolute ethanol, potassium dichromate and Phloroglucinol solution may be used. *Candida shehatae* may be chosen as the microorganism. The organism needs to be maintained on Sabouraud Dextrose Agar (10 g/L neo-peptone, 20 g/L dextrose, pH 6.5; SDA) at 4 °C. Subculturing of it in Sabouraud xylose agar (SXA) medium containing 20 g/L xylose will be useful for fermentation [10].

2.2. Preparation of water hyacinth

Fresh water hyacinth with long stem is used for the experiment. The water hyacinth must be washed thoroughly for several times with tap water to remove adhering dirt, and then chopped into small pieces (~2–2.5 cm), blended to small particles (~4–5 mm), and finally dried in a hot air oven at 100–105 °C for 5–6 h. The dried material needs to be stored at room temperature until used. The preparation of water hyacinth as a raw material for ethanol conversion is described in Fig. 2.

2.3. Pretreatment

The following processes can be used for pretreatment of water hyacinth:

2.3.1. Steam explosion

Combined chemical and physical treatment systems are necessary for dissolving hemicellulose and alteration of lignin structure, providing an improved accessibility of the cellulose for hydrolytic enzymes. Research shows that the most successful physicochemical pretreatments include thermochemical treatments such as steam explosion [11]. In this process, chipped water hyacinth needs to be treated with high-pressure saturated steam, and then the pressure has to be swiftly reduced, making the materials undergo an explosive decompression. Steam explosion has to be initiated at a temperature of 160–260 °C at a pressure of 0.69–4.83 MPa for

several seconds to a few minutes before the material get exposed to atmospheric pressure. The processes cause hemicellulose degradation and lignin transformation at high temperature, thus increasing the potential of cellulose hydrolysis. Addition of H₂SO₄, SO₂ or CO₂ in steam explosion of lignocellulose can effectively improve enzymatic hydrolysis, and lead to more complete liquefaction of hemicellulose, glucan, xylan, mannan, galactan, and arabinan [11,12].

2.3.2. Acid hydrolysis

Water hyacinth needs to be hydrolyzed using different acids to produce xylose, arabinose, glucose, and acetic acid by cleavage of the β-1,4 linkages of glucose or xylose monomers, acetyl groups. The overall fermentable sugar available by acid hydrolysis may be 90% of the theoretical value of the sugar present [13,14]. Dilute acid process has to be conducted under temperatures of 120–200 °C and pressures of 103 kPa (15 psi) to 517 kPa (75 psi), and have reaction times in the range of 30 min to 2 h by continuous processes [5,15]. The concentrated acid processes may be successful, for producing higher yields of sugar. This process typically involves the use of 60–90% sulfuric acid, mild temperatures, and moderate pressures created by pumping materials from one vessel to another vessel for effective hydrolysis. The primary advantage of the concentrated acid process is the high sugar recovery efficiency, which can be on the order of >90% for both xylose and glucose sugars [5].

Acid hydrolysis processes have several disadvantages due to formation of toxic compounds, such as furfural, hydroxyl-methyl furfural, acetic acid, formic acid, levulinic acid, etc., which inhibit the fermentation. Removal of these compounds causes additional costs. The use of lime to neutralize acid has the disadvantage of significant loss of sugar in the form of gypsum. However, such processes could be replaced by highly economical chromatographic separations with acid recycling.

2.3.3. Alkali pretreatment

Effect of alkaline pretreatment depends on the lignin content of the materials. Some bases may be used for pretreatment of water hyacinth. The mechanism of alkaline hydrolysis is saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components, for example, lignin and other hemicellulose. The porosity of the lignocellulosic materials increases with the removal of the crosslinks. Dilute NaOH (0.5%) treatment of lignocellulosic materials causes swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure. Ammonia was also used for the pretreatment to re-move lignin. The efficiency of delignification was 50–70% for water hyacinth.

2.3.4. Biological pretreatment

Biological treatment involves the use of whole organisms or enzymes in pretreatment of water hyacinth. Both fungi and bacteria may be used for treatment of water hyacinth. Fungal pretreatment of lignocellulose is a new method for improvement of digestibility [16]. White-, brown- and soft-rot fungi are generally used to degrade lignin and hemicellulose in water hyacinth whereby brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials [11]. Recent studies have shown that *Aspergillus terreus* [17], *Trichoderma* sp. [18], *Cyathus stercoreus* [19], *Lentinus squarrosulus* [20], *Penicillium camemberti* [21], grown at 25–35 °C for 3–22 days resulted to 45–75% and 65–80% holocellulose and lignin degradation, respectively. Recombinant strains of *S. cerevisiae* may be genetically engineered to carry out simultaneous saccharification and fermentation (SSF) to produce extracellular endoglucanase

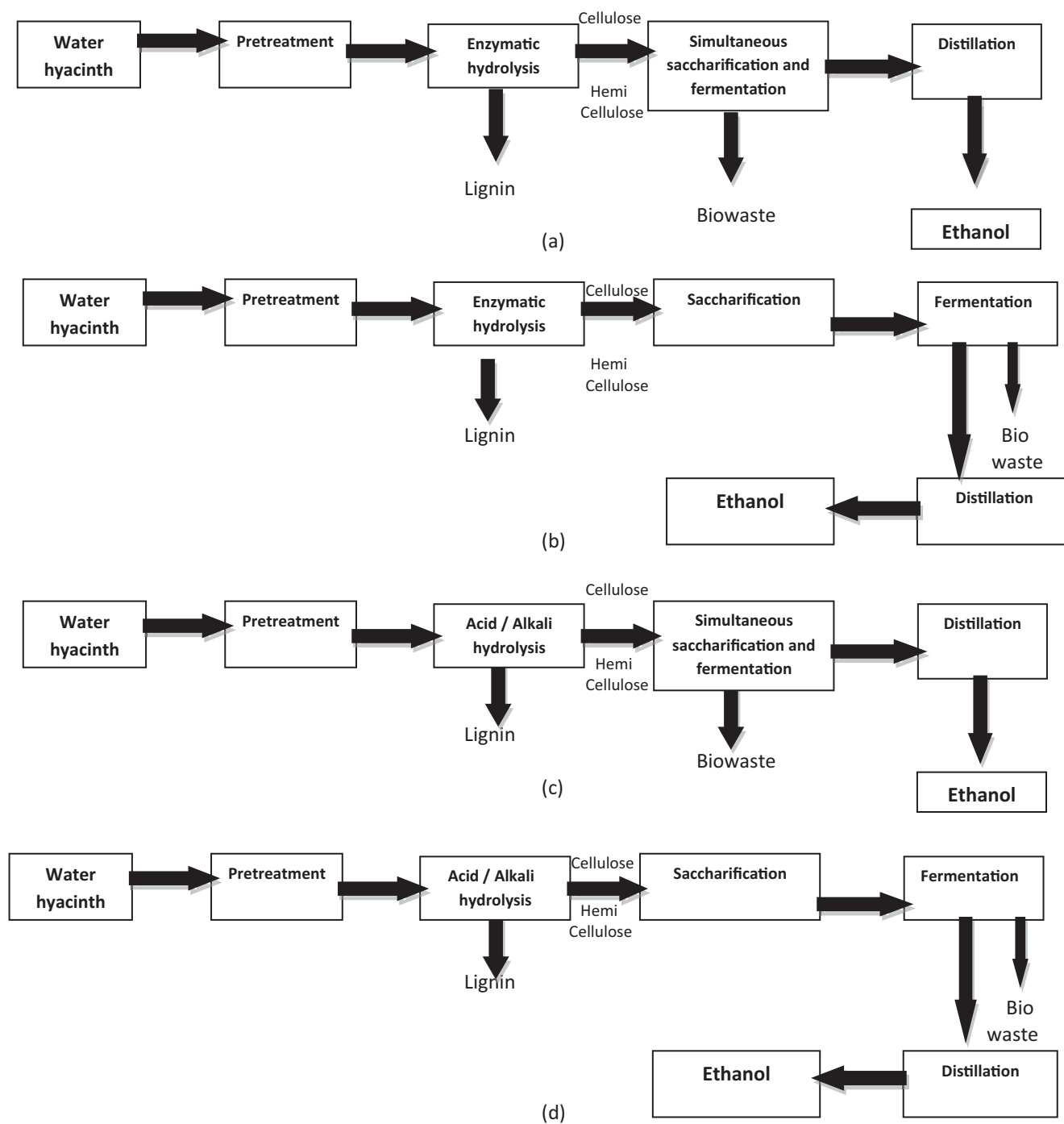


Fig. 1. General description of the process.

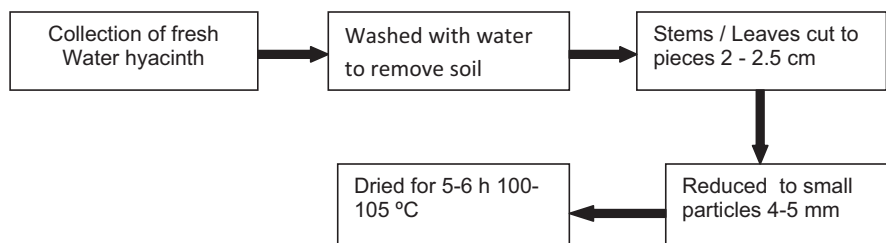


Fig. 2. Preparation of water hyacinth.

and β -glucosidase that ferment cellulose and hemicellulose to 6-carbon and 5-carbon sugars and subsequent fermentation to ethanol [22–26].

Bacterial pretreatment of lignocellulose involves both anaerobic and aerobic systems. Anaerobic degradation utilizes mainly mesophilic, rumen derived bacteria [27–31]. Meanwhile, in aerobic system, actinomycete *Streptomyces griseus* produce high levels of extracellular hydrolytic enzyme that degrade lignocellulose [32]. *Escherichia coli* and *Klebsiella oxytoca* strains may be genetically engineered to produce microbial biocatalysts that produce bioethanol from lignocellulosic materials [33,34]. Three major groups of cellulases is involved in the hydrolysis process: (1) endoglucanase (endo-1,4-glucanohydrolase) attacks regions of low crystallinity in the cellulose fiber, creating free chain-ends; (2) exoglucanase or cellobiohydrolase (CBH), 1,4- β -glucan cellobiohydrolase degrades the molecule further by removing cellobiose units from the free chain-ends and (3) β -glucosidase hydrolyzes cellobiose to produce glucose [11]. A number of ancillary enzymes attack hemicellulose, such as glucuronidase, acetylsterase, feruloylsterase, xylanase, β -xylosidase, galactomannanase and glucomannanase [35–40]. Ligninolytic enzymes

primarily involve in lignin degradation in oxidative reactions. The main enzyme involved is lignin peroxidase, manganese peroxidase and laccase [41–44].

2.4. Preparation of hemicellulose acid hydrolysate

One hundred grams of dried water hyacinth is generally mixed with 1% or 10% of sulfuric acid to a final volume of 1000 mL. The mixture was autoclaved at 121 °C, 103 kPa for 15 min and cooled down to room temperature. The hydrolysate will then be filtered using Whatman paper No. 1 to remove the unhydrolyzed material. The filtrate may finally be collected and the xylose content was analysed [10].

2.4.1. Detoxification of hemicellulose hydrolysate

The hemicellulose acid hydrolysate needs to be heated to 60 °C and then basified with solid NaOH until the pH reaches 9.0–9.5. Solid $\text{Ca}(\text{OH})_2$ was added to the solution in order to detoxify harmful materials that are present in the hydrolysate. After removal of insoluble residues by filtration, the supernatant should be collected for further use as fermentable sugars [10].

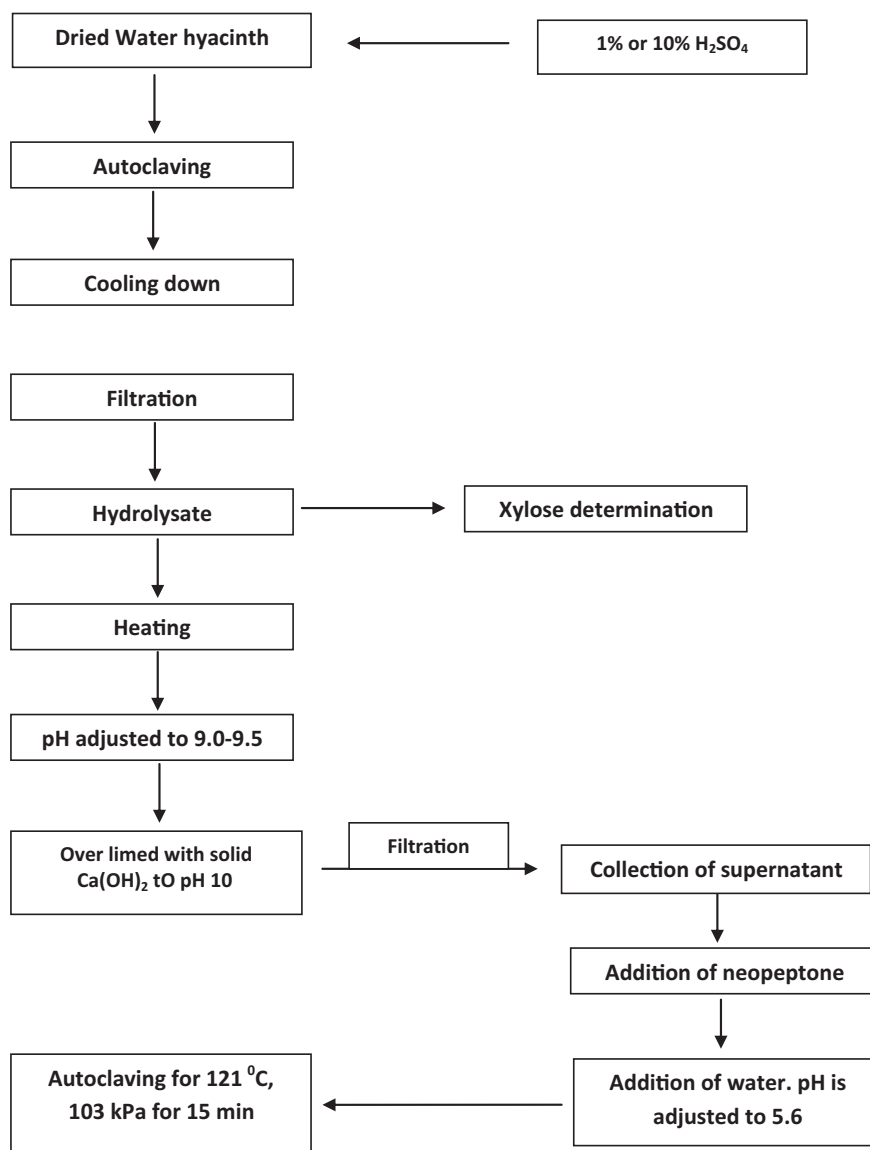


Fig. 3. Hydrolysis, detoxification and media preparation.

2.5. Lignin separation

2.5.1. Preparation of the lignocellulosic hydrolysate

The pretreated material after being washed with water may be properly filtered and the filtrate may then be collected for sugar analysis.

2.5.2. Detoxification procedure

The method for enzymatic detoxification includes samples treated with laccase (1 M), lignin peroxidase (1 M), laccase and lignin peroxidase combined (1 M of each), and the control (water added instead of enzyme). This sample needs to be analysed for fermentability. This sample needs to be analysed for fermentability by GC–MS; their phenolic compound content can be quantified, by gel-permeation chromatography. The hydrolysate may then be adjusted with NaOH to pH 5.3 for samples intended for controls and for treatment with laccase, to pH 3.2 for treatment with lignin peroxidase, and to pH 4.5 for combined treatment with laccase and lignin peroxidase. Laccase, lignin peroxidase or water may be added as required. All samples may then be incubated at 30 °C for 12 h in a rotary shaker (90 rpm). Hydrogen peroxide 0.2 M needs to be added every hour to the samples containing peroxidase and at the end of the experiment; catalase (0.04 mg/mL) may be added [45] as shown in Fig. 3.

2.6. Fermentation

2.6.1. Use of *Pichia stipitis* and *C. shehatae*

The fermentation organism must be able to ferment all monosaccharides present and in addition, withstand potential inhibitors in the hydrolysates. The most commonly used ethanol producer, *S. cerevisiae*, cannot ferment pentoses, which may constitute up to 40% of the raw material. Among the xylose fermenting yeasts *P. stipitis* has shown promise for industrial applications, because it ferments xylose rapidly with a high ethanol yield and apparently produces no xylitol [46] and is able to ferment a wide range of sugars (including cellobiose) than *C. shehatae* [47].

The ability of *P. stipitis* and *C. shehatae* yeasts to ferment xylose efficiently to ethanol has received widespread attention and led to many investigations which use the lignocellulosic hydrolysate as fermentable substrate. There is huge scope of research in this direction with different microorganisms to develop a standard procedure for fermentation of the biomass requiring less time.

2.7. Yield and efficiency

The production yields of ethanol obtained from the acid hydrolysis of water hyacinth using different approaches has been studied. Some results revealed that using the sulfuric hydrolysis following by the bioconversion of *C. shehatae* yielded ethanol with the maximum content of 1.01 g/L, the maximum yield coefficient of 0.19 g g⁻¹ and the productivity of 0.008 g L⁻¹ h⁻¹ [10]. These values are as well comparable to those obtained from the phenol-tolerant strain of xylose fermenting bacterium [48]. This report herein showed only 1.8 fold lower yield coefficient than those obtained from using the fully equipped fermentor [49]. Therefore, further optimization of the development of a versatile tool for ethanol production should become the objective of further research.

3. Conclusion

Water hyacinth is one of the worst weeds causing the major problem to the aquatic ecosystem particularly in the tropics. Although measures to control its population have been widely applied including use of herbicides and mechanical removal, in most of the cases, it remains ineffective due to the pernicious

invasive growing of the aquatic hyacinth. The technique described above helps lowering the plant population while providing a simple and low-cost process that is suitable to the developing countries; where the cellulose portions of the herbs are hydrolyzed by enzymes into glucose sugar that are fermented to bioethanol. It is found that 1 kg of cellulose yields 1.1 kg of glucose and 1 kg of cellulose yield 0.56 kg of ethanol. The sugars from the hemicellulose are also fermented to bioethanol. Many studies were done about pretreatment process. A number of methods were developed for this purpose – focusing on physical, chemical or biological processes. To get Green Ethanol, we must concentrate on biological process for pretreatment and fermentation. The main issue in biological process is that it is time consuming. However, researchers are working on reduction of the time factor. As biological process is eco-friendly and leads to energy savings, we must look through to the biological process to preserve the environment and protect our planet which is increasingly under threat.

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References

- [1] Bentley RW. Global oil and gas depletion: an overview. *Energy Policy* 2002;30:189–205.
- [2] Cavallo AJ. Predicting the peak in world oil production. *Nat Resour Res* 2002;11:187–95.
- [3] Demirbas A. Products from lignocellulosic materials via degradation processes. *Energy Sources A: Recov Util Environ Effects* 2008;30(1):27–37.
- [4] Takahashi CM, Lima KGC, Takahashi DF, Alterthum F. *World J Microbiol Biotechnol* 2000;16:829, doi:10.1023/A:1008987103701.
- [5] Badger PC. In: Ja-nick J, Whipkey A, editors. *Trends in New Crops and New Uses*. Alexandria, VA: ASHS Press; 2002.
- [6] Mielenz JR. *Curr Opin Microbiol* 2001;4(3):324, doi:10.1016/S1369-5274(00)00211-3.
- [7] Dhamija SS, Sangwan S. Industrial Production of Ethanol in India. Hisar, Haryana 125004: Department of Microbiology, College of Basic Sciences & Humanities, CCS HAU; 2006.
- [8] Knauf M, Moniruzzaman M. *Int Sugar J* 2004;106(1263):147.
- [9] Malik A. Environmental challenge vis a vis opportunity: the case of water hyacinth. *Environ Int* 2007;33:122–38.
- [10] Isarankura-Na-Ayudhya C, Tantimongkolwat T, Kongpanpee T, Prabhate P, Prachayasittikul V. Appropriate technology for the bio-conversion of water hyacinth (*Eichhornia crassipes*) to liquid ethanol: future prospects for community strengthening and sustainable development. *EXCLI J* 2007;6:167–76. ISSN 1611-2156.
- [11] Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour Technol* 2002;83(1):1–11, doi:10.1016/S0960-8524(01)00212-7.
- [12] Jeoh T, Agblevor FA. Characterization and fermentation of steam exploded cotton gin waste. *Biomass Bioenergy* 2001;21(2):109–20, doi:10.1016/S0961-9534(01)00028-9.
- [13] Lavarack BP, Griffin GJ, Rodman D. *Biomass Bioenergy* 2002;23(5):367, doi:10.1016/S0961-9534(02)00066-1.
- [14] Frederick Jr WJ, Lien SJ, Courchene CE, DeMartini NA, Ragauskas AJ. *Bioresour Technol* 2008;99(11):5051, doi:10.1016/j.biortech.2007.08.086.
- [15] Kim KH, Tucker MP, Nguyen QA. *Biotechnol Prog* 2002;18(3):489, doi:10.1021/bp025503i.
- [16] Sinegani AAS, Emtiazi G, Hajrasulhi S, Shariatmadari H. Biodegradation of some agricultural residues by fungi in agitated submerged cultures. *Afr J Biotechnol* 2005;10:1058–61.
- [17] Emtiazi G, Naghavi N, Bordbar A. Biodegradation of lignocellulosic waste by *Aspergillus terreus*. *Biodegradation* 2001;12(4):257–61, doi:10.1023/A:1013155621336.
- [18] Pérez J, Muñoz-Dorado J, De La Rubia T, Martínez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *J Int Microbiol* 2002;5(2):53–63, doi:10.1007/s10123-002-0062-3.
- [19] Keller FA, Hamilton JE, Nguyen QA. Microbial pretreatment of biomass: potential for reducing severity of thermochemical biomass pretreatment. *J Appl Biochem Biotechnol* 2003;105(1–3):27–41, doi:10.1385/ABAB:105:1-3:27.
- [20] Shide EG, Wyup PA, Nok A. Studies on the degradation of wood sawdust by *Leptinus squarrosulus* (Mont.) Singer. *Afr J Biotechnol* 2004;3(8):395–8.

- [21] Taseli BK. Fungal treatment of hemp-based pulp and paper mill wastes. *Afr J Biotechnol* 2008;7(3):286–9.
- [22] Sedlak M, Ho NWY. Production of ethanol from cellulosic biomass hydrolysates using genetically engineered *Saccharomyces* yeast capable of co fermenting glucose and xylose. *J Appl Biochem Biotechnol* 2004;114(1–3):403–16, doi:10.1385/ABAB:114:1–3:403.
- [23] Van Maris AJA, Abbott DA, Bellissimi E, Van Den Brink J, Kuyper M, Luttik MAH, et al. Alcoholic fermentation of carbon sources in biomass hydrolysates by *Saccharomyces cerevisiae*: current status. *Antonie Van Leeuwenhoek* 2006;90(4):391–418, doi:10.1007/s10482-006-9085-7.
- [24] Haan RD, Rose SH, Lynd LR, Van Zyl WH. Hydrolysis and fermentation of amorphous cellulose by recombinant *Saccharomyces cerevisiae*. *Metab Eng* 2007;9(1):87–94, doi:10.1016/j.ymben.2006.08.005.
- [25] Chu BCH, Lee H. Genetic improvement of *Saccharomyces cerevisiae* for xylose fermentation. *Biotechnol Adv* 2007;25(5):425–41, doi:10.1016/j.biotechadv.2007.04.001.
- [26] Wisselink HW, Toirkens MJ, Berriel MDRF, Winkler AA, Van Dijken JP, Pronk JT, et al. Engineering of *Saccharomyces cerevisiae* for efficient anaerobic alcoholic fermentation of L-arabinose. *Appl Environ Microbiol* 2007;15:4881–91, doi:10.1128/AEM.00177-07.
- [27] Han SK, Shin HS. Enhanced acidogenic fermentation of food waste in a continuous-flow reactor. *Waste Manage Res* 2002;20(2):110–8.
- [28] Hu ZH, Yu HQ. Application of rumen microorganisms for enhanced anaerobic fermentation of corn stover. *Process Biochem* 2005;40(7):2371–7, doi:10.1016/j.procbio.2004.09.021.
- [29] Neves L, Oliveira R, Alves MM. Anaerobic co-digestion of coffee waste and sewage sludge. *Waste Manage* 2006;26(2):176–81, doi:10.1016/j.wasman.2004.12.022.
- [30] Hu ZH, Liu SY, Yue ZB, Yan LF, Yang MT, Yu HQ. Microscale analysis of in vitro anaerobic degradation of lignocellulosic wastes by rumen microorganisms. *Environ Sci Technol* 2008;42(1):276–81, doi:10.1021/es071915h.
- [31] Yue ZB, Yu HQ, Hu ZH, Harada H, Li YY. Surfactant-enhanced anaerobic acidogenesis of *Canna indica* L. by rumen cultures. *Bioresour Technol* 2008;99(9):3418–23, doi:10.1016/j.biortech.2007.08.010.
- [32] Arora A, Nain L, Gupta JK. Solid-state fermentation of wood residues by *Streptomyces griseus* B1, a soil isolate, and solubilization of lignin. *World J Microbiol Biotechnol* 2005;21(3):303–8, doi:10.1007/s11274-004-3827-3.
- [33] Jarboe LR, Grabar TB, Yomano LP, Shanmugan KT, Ingram LO. Development of ethanologenic bacteria. *Adv Biochem Eng Biotechnol* 2007;108:237–61, doi:10.1007/10.2007.068.
- [34] Peterson JD, Ingram LO. Anaerobic respiration in engineered *Escherichia coli* with an internal electron acceptor to produce fuel ethanol. *Ann NY Acad Sci* 2008;1125:363–72.
- [35] Nikolov T, Bakalova N, Petrova S, Benadova R, Spasov S, Kolev D. An effective method for bioconversion of delignified waste-cellulose fibers from the paper industry with a cellulase complex. *Bioresour Technol* 2000;71(1):1–4, doi:10.1016/S0960-8524(99)00059-0.
- [36] Draude KM, Kurniawan CB, Duff JB. Effect of oxygen delignification on the rate and extent of enzymatic hydrolysis of lignocellulosic material. *Bioresour Technol* 2001;79(2):113–20, doi:10.1016/S0960-8524(01)00055-4.
- [37] Aranda E, Sampedro I, Ocampo JA, García-Romera I. Contribution of hydrolytic enzymes produced by saprophytic fungi to the decrease in plant toxicity caused by water-soluble substances in olive mill dry residue. *J Appl Microbiol Biotechnol* 2004;64(1):132–5, doi:10.1007/s00253-003-1368-6.
- [38] Mtui G, Nakamura Y. Bioconversion of lignocellulosic waste from selected dumping sites in Dar es Salaam. *Tanzania Biodegrad* 2005;16(6):493–9, doi:10.1007/s10532-004-5826-3.
- [39] Roman HJ, Burgess JE, Pletschke BI. Enzyme treatment to decrease solids and improve digestion of primary sewage sludge. *Afr J Biotechnol* 2006;5(10):963–7.
- [40] Georgieva TI, Hou X, Hilstrom T, Ahring BK. Enzymatic hydrolysis and ethanol fermentation of high dry matter wet-exploded wheat straw at low enzyme loading. *J Appl Biochem Biotechnol* 2008;148(1–3):35–44, doi:10.1007/s12010-007-8085-z.
- [41] Hao JJ, Tian XJ, Song FQ, He XB, Zhang ZJ, Zhang P. Involvement of lignocellulolytic enzymes in the decomposition of leaf litter in a subtropical forest. *J Eukaryot Microbiol* 2006;53(3):193–8.
- [42] Mtui G, Nakamura Y. Characterization of lignocellulosic enzymes from white-rot fungus *Phlebia cryocreas* isolated from a marine habitat. *J Eng Appl Sci* 2007;2:1501–8.
- [43] Mtui G, Nakamura Y. Lignocellulosic enzymes from *Flavodon flavus*, a fungus isolated from Western Indian Ocean off the Coast of Dar es Salaam, Tanzania. *Afr J Biotechnol* 2008;7(17):3066–72.
- [44] Mtui G, Masalu R. Extracellular enzymes from Brown-rot fungus *Laetioporus sulphureus* isolated from mangrove forests of Coastal Tanzania. *Sci Res Essay* 2008;3:154–61.
- [45] Jönsson LJ, Palmqvist E, Nilvebrant N-O, Hahn-Hägerdal B. Detoxification of wood hydrolysates with laccase and peroxidase from the white-rot fungus *Trametes versicolor*. *Appl Microbiol Biotechnol* 1998;49:691–7.
- [46] Dominguez H, Nunez MJ, Chamy R, Lema J. Determination of kinetic parameters of fermentation processes by a continuous unsteady-state method: application to the alcoholic fermentation of D-xylose by *Pichia stipitis*. *Biotechnol Bioeng* 1993;41:1129–32.
- [47] Du Preez JC, Bosch M, Prior BA. The fermentation of hexose and pentose sugars by *Candida shehatae* and *Pichia stipitis*. *Appl Microbiol Biotechnol* 1986;23:228–33.
- [48] Asli AE, Boles E, Hollenberg CP, Errami M. Conversion of xylose to ethanol by a novel phenol tolerant strain of *Enterobacteriaceae*, isolated from olive mill wastewater. *Biotechnol Lett* 2002;24:1101–5.
- [49] Nigam JN. Bioconversion of water hyacinth (*Eichhornia crassipes*) hemicellulose acid hydrolysate to motor fuel ethanol by xylose-fermenting yeast. *J Biotechnol* 2002;97:107–11.